PB86-177789

Inactivation of 'Campylobacter jejuni' by Chlorine and Monochloramine

(U.S.) Environmental Protection Agency
Cincinnati, OH

1986

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RI PB86-177789
EPA/600/J-86/017		

4. TITLE AND SUBTITLE
INACTIVATION OF CAMPYLOBACTER JEJUNI BY CHLORINE AND MONOCHLORAMINE (Journal Version)

5. REPORT DATE
1986

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
M.J. Blaser,[1] P.F. Smith,[2], W.L. Wang,[2] and J.C. Hoff[3]

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
[1]Univ. Colorado School of Medicine, Denver CO 80220
[2]Veterans Adminis. Medical Center, Denver, CO 80220
[3]U.S. Environmental Protection Agency, Cincinnati, OH 45268

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.
CR-810169 byproduct

12. SPONSORING AGENCY NAME AND ADDRESS
Water Engineering Research Laboratory-Cincinnati, OH
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

13. TYPE OF REPORT AND PERIOD COVERED
Journal Article

14. SPONSORING AGENCY CODE
EPA/600/14

15. SUPPLEMENTARY NOTES
Project Officer, J.C. Hoff, 513-569-7331
Applied and Environmental Microbiology, 51(2):307-311, Feb. 1986

16. ABSTRACT

Campylobacter jejuni and closely related organisms are important bacterial causes of acute diarrheal illness in the United States. Both endemic and epidemic infections have been associated with consuming untreated or improperly treated surface water. The susceptibility of three C. jejuni strains and Escherichia coli ATCC 11229 were compared with standard procedures used to disinfect water. The results suggest that disinfection procedures commonly used for treatment of drinking water to remove coliform bacteria are adequate to eliminate C. jejuni and further correlate with the absence of outbreaks associated with properly treated water.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	6
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA/600/J-86/017
JOURNAL ARTICLE

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1986, p. 307–311
0099-2240/86/020307-05$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 51, No. 2

Inactivation of *Campylobacter jejuni* by Chlorine and Monochloramine

MARTIN J. BLASER,[1,2]* PAUL F. SMITH,[2] WEN-LAN L. WANG,[3] AND JOHN C. HOFF[4]

Division of Infectious Diseases, Department of Medicine, University of Colorado School of Medicine,[1] and Infectious Disease Section[2] and Microbiology Laboratory,[3] Veterans Administration Medical Center, Denver, Colorado 80220, and Drinking Water Research Division, Environmental Protection Agency, Cincinnati, Ohio 45268[4]

Received 6 June 1985/Accepted 23 November 1985

Campylobacter jejuni **and closely related organisms are important bacterial causes of acute diarrheal illness in the United States. Both endemic and epidemic infections have been associated with consuming untreated or improperly treated surface water. We compared susceptibility of three *C. jejuni* strains and *Escherichia coli* ATCC 11229 with standard procedures used to disinfect water. Inactivation of bacterial preparations with 0.1 mg of chlorine and 1.0 mg of monochloramine per liter was determined at pH 6 and 8 and at 4 and 25°C. Under virtually every condition tested, each of the three *C. jejuni* strains was more susceptible than the *E. coli* control strain, with greater than 99% inactivation after 15 min of contact with 1.0 mg of monochloramine per liter or 5 min of contact with 0.1 mg of free chlorine per liter. Results of experiments in which an antibiotic-containing medium was used suggest that a high proportion of the remaining cells were injured. An animal-passaged *C. jejuni* strain was as susceptible to chlorine disinfection as were laboratory-passaged strains. These results suggest that disinfection procedures commonly used for treatment of drinking water to remove coliform bacteria are adequate to eliminate *C. jejuni* and further correlate with the absence of outbreaks associated with properly treated water.**

Campylobacter jejuni and closely related species are the most common bacterial causes of acute diarrheal illnesses in the United States and many other parts of the world (5–7). These organisms reside in the intestinal tracts of a wide variety of wild and domesticated birds and mammals (6). From these sources, transmission to humans occurs via contaminated water, as well as other modes. Since 1978, seven waterborne outbreaks of *Campylobacter* enteritis have been reported, and endemic waterborne campylobacteriosis also occurs (6, 17).

In virtually every report, diarrheal illness was associated with drinking untreated or inadequately treated water (6). However, because of the frequency with which waterborne *Campylobacter* infection occurs and because source waters may be contaminated with these organisms, it is important to assess their susceptibility to common water disinfection methods. Chlorine, which is widely used for water disinfection, rapidly inactivates a variety of enteric pathogens (2, 9–11, 15). We previously have shown that three strains of *C. jejuni* are inactivated by chlorine, but the conditions studied most nearly resembled those in hospitals and laboratories (18). In a preliminary report assessing susceptibility of campylobacters to chlorine under conditions similar to those found during routine water treatment, we also found significant inactivation, but the system studied was not chlorine demand free (8).

The purpose of the present study was to evaluate whether chlorine or monochloramine would inactivate *C. jejuni* under chlorine demand-free conditions that simulate those found during routine water treatment. Comparative inactivation studies also were conducted with an indicator strain, *Escherichia coli* ATCC 11229.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. The *C. jejuni* strains used in these studies, originally isolated from patients with diarrhea, were the PEN 1, PEN 2, and PEN 3 type strains in the *Campylobacter* serotyping system based on heat-stable antigens (14). These represent three of the most common serotypes of *C. jejuni* isolated from humans (14). These isolates had been passaged multiple times on artificial media before use in this study. To obtain a less-passaged strain, an adult mouse was infected with PEN 1 as previously described (3). After intestinal tract colonization occurred, stool specimens were placed into brucella broth (BBL Microbiology Systems, Cockeysville, Md.), and the contents were vortex mixed. A 0.1-ml sample of the suspension was pipetted onto a *Campylobacter* blood agar plate (CBAP; BBL) and streaked for isolation, and the plate was incubated at 42°C for 24 h in an atmosphere containing 85% N_2, 10% CO_2, and 5% O_2. This medium contains the following antimicrobial agent concentrations per liter: vancomycin, 10 mg; trimethoprim, 5 mg; polymyxin B, 2,500 IU; amphotericin B, 2 mg. After incubation, *C. jejuni* colonies were subcultured onto Trypticase (BBL) soy plates with 5% sheep blood (BAP; PASCO, Wheat Ridge, Colo.), and the incubation procedure was repeated. At the end of the second incubation period, isolated PEN 1 colonies were recovered from this plate and used in disinfection experiments. When we found that susceptibility was nearly identical for animal-passaged and the laboratory-passaged PEN 1 isolates, we used the latter and the laboratory-passaged PEN 2 and PEN 3 strains in subsequent assays. For comparative studies, we used *E. coli* ATCC 11229, which was subcultured on the same BAP.

Preparation of chlorine test solutions. The glassware was thoroughly cleaned and rinsed with membrane-filtered (Millipore Corp., Bedford, Mass.), deionized water. Stock chlorine solutions were prepared from a 5% solution of sodium

hypochlorite (J. T. Baker Chemical Co., Phillipsburg, N.J.). Chlorine demand-free water was prepared by adding the hypochlorite solution to deionized water to a final chlorine concentration of 5 mg/liter. The chlorinated water was allowed to stand at room temperature in direct sunlight for at least 1 week before use. All buffers were prepared with chlorine demand-free water. The buffer system for the inactivation experiments consisted of a 0.05 M phosphate buffer (NaH_2PO_4-Na_2HPO_4) prepared at pH 6 or 8. Buffers were considered chlorine demand-free and suitable for use when the chlorine concentration was less than 0.01 mg/liter as titrated amperometrically with a Wallace and Tiernan titrimeter (1).

Preparation of monochloramine test solutions. Monochloramine was made by adding sodium hypochlorite to phosphate buffer (0.05 M; pH 6 or 8) to a concentration of 1 mg/liter. Ammonium sulphate was added to this solution in a final concentration of 5 mg/liter. This solution was then allowed to equilibrate at the test temperature for 1 h before measurement of free and total chlorine and use in the disinfection assay. The resulting solution produced a monochloramine concentration of 1.0 mg/liter with a free chlorine concentration less than 0.05 mg/liter. Because this method left a residual ammonium sulfate level of 4.0 mg/liter, we determined whether that would inactivate *C. jejuni* and found no significant killing.

Chlorine determinations. The free residual chlorine content of all stock and treatment solutions was determined by amperometric titration (1). The chlorine residual of treatment and control solutions immediately before and after each treatment experiment was determined by the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method (1).

Effect of sodium thiosulfate on *C. jejuni* and *E. coli*. All water used was chlorine demand free. To neutralize the effect of chlorine, an autoclaved 1.0% solution of sodium thiosulfate (Sigma Chemical Co., St. Louis, Mo.) was used. *C. jejuni* PEN 1 cells grown for 36 h at 42°C in the microaerobic atmosphere, or *E. coli* cells grown for 18 h at 37°C in ambient atmosphere were harvested from BAP in 10.0 ml of water by gentle scraping with a sterile rayon-tipped swab. The cell suspensions were pelleted by centrifugation at 5,000 × *g* for 10 min, and the pellet was washed three times in water. Tenfold serial dilutions of the suspensions were made in water, and initial cell counts were made by plating in triplicate 0.1 ml of the appropriate dilution. To obtain equal numbers, 1.0 ml each of the 10^{-5} dilution of the original *E. coli* suspension and the 10^{-3} dilution of the original *C. jejuni* suspension was inoculated into 9.0 ml of water (control) or 9.0 ml of a 1.0% solution of sodium thiosulfate in phosphate-buffered water (pH 7.0) for 60, 120, or 180 min at 5°C. Serial 10-fold dilutions of the reaction mixtures were made at the end of each incubation in water. Counts were made by plating 0.1 ml of each dilution in triplicate as described above.

Experimental procedure. Twenty-four-hour cultures of *C. jejuni* strains and 4-h cultures of *E. coli* were used for these experiments. The bacterial cells were harvested, prepared, and enumerated as described above. In the standard assay, 99 ml of the chlorine or monochloramine solution in buffer was placed in each of nine sterile, acid-cleaned milk dilution bottles. To each of three bottles was added 1 ml of the appropriate suspension of *E. coli* or *C. jejuni* in buffer to a final concentration of 10^3 to 10^5 CFU/ml; three bottles received buffer alone without bacterial cells. In parallel, bottles with buffer only but no chlorine or monochloramine were inoculated with the bacterial suspensions to monitor for nonspecific cell death. Assays were done at 4 or 25°C and at pH 6 or 8. After predetermined incubation times, 1.0 ml was removed from the reaction mixtures and added to 1.0 ml of sterile 1% sodium thiosulfate in water. The suspensions then were diluted in water, spread plated onto duplicate BAP, and incubated as described above, and colonies were counted. In a series of experiments, treated suspensions were plated in parallel onto nonselective BAP and selective CBAPs. Previous studies have demonstrated that this medium is not inhibitory to *C. jejuni* (19).

Experiments with *C. jejuni* in fresh fecal pellets. To assess whether laboratory-passaged *C. jejuni* had susceptibility similar to that of organisms directly from animals, we conducted the following experiment. Five female Naval Medical Research Institute (NMRI) mice were chronically colonized with *C. jejuni* PEN 1 as previously described (3). One day before disinfection assays were to be done, mice were given a further oral dose of PEN 1 as previously described (3). The following day, feces from all five mice were pooled in 20 ml of sterile distilled water and vortex mixed to homogenicity. Because most chlorine demand in feces is due to the nonbacterial phase, we concentrated the bacterial pellet. Centrifugation at 60 × *g* for 20 min and 270 × *g* for 20 min pelleted large debris from the suspension. Centrifugation at 2,400 × *g* for 20 min then pelleted the bacterial phase. This pellet was suspended in sterile distilled water, and 10-fold dilutions were plated onto BAP incubated at 37°C in ambient atmosphere to detect aerobic fecal flora and onto CBAP incubated at 42°C under microaerobic conditions to detect *C. jejuni*. Disinfection assays were done as described above, except that the initial free chlorine concentration was 0.45 to 0.48 mg/liter to overcome the demand of the fecal bacterial pellet.

RESULTS

Interaction of test strains with thiosulfate. The initial studies done were to determine whether the sodium thiosulfate neutralizer in the concentration used (1.0%) was inhibitory to any of the test strains. Neither *C. jejuni* PEN 1 nor the *E. coli* strain was significantly inhibited during a 180-min test incubation (Fig. 1). During experimental disinfection assays, the maximum period of contact with thiosulfate was less than 30 min, well within the period of no inhibition.

Chlorine disinfection. Samples of water or buffers only were used in the chlorine or monochloramine assays if chlorine demand was less than 0.01 mg/liter as determined by amperometric titration. Chlorine demand produced by inoculation of test bacterial cells into the assay mixture ranged from 0.04 to 0.06 mg/liter as determined by the DPD method and mostly occurred during the first minute. For both the *E. coli* and *C. jejuni* strains, disinfection was more rapid at pH 6 than at pH 8 (Table 1), reflecting the higher expected concentrations of the more bactericidal HOCl at the lower pH. Disinfection proceeded similarly at 4 and 25°C. For the *C. jejuni* strains, greater than 99% (2 $\log_{10}$) inactivation occurred within 5 min of contact with 0.1 mg of free chlorine per liter under every condition studied; for most conditions, greater than 99% inactivation occurred within 30 s. Under most conditions shown, the *C. jejuni* strains were as susceptible to chlorine disinfection as was the *E. coli* test strain or more so. There were few differences among the three *C. jejuni* strains. In studies not shown, free chlorine levels of 0.25 mg/liter produced complete inactivation of *C. jejuni* at the first time point studied (30 s), whereas contact with the buffer solutions alone produced no significant inactivation during the entire experiment.

TABLE 1. Effect of chlorine (0.1 mg/liter) on three *C. jejuni* strains and *E. coli* ATCC 11229

Temp, pH, and strain	Initial bacterial count (log_{10} CFU/ml)	−(Log_{10} surviving fraction)[a] at:		
		30 s	1 min	5 min
25°C, pH 6				
E. coli ATCC 11229	5.23[a]	2.28	3.54	3.39
C. jejuni PEN 1	4.49	1.41	2.21	2.04
C. jejuni PEN 2	3.88	2.58	2.88	2.88
C. jejuni PEN 3	5.50	>4.50[b]	4.02	>4.50
25°C, 8				
E. coli ATCC 11229	5.22	0.90	1.55	3.73
C. jejuni PEN 1	4.46	0.78	0.86	2.08
C. jejuni PEN 2	3.93	>2.93	>2.93	>2.93
C. jejuni PEN 3	5.50	0.64	0.63	3.45
4°C, pH 6				
E. coli ATCC 11229	5.49	2.70	3.53	3.89
C. jejuni PEN 1	5.12	>4.12	>4.12	>4.12
C. jejuni PEN 2	5.60	3.70	4.12	>4.60
C. jejuni PEN 3	5.70	>4.70	>4.70	>4.70
4°C, pH 8				
E. coli ATCC 11229	5.47	1.15	2.63	4.01
C. jejuni PEN 1	5.02	ND[c]	3.54	>4.02
C. jejuni PEN 2	5.60	2.15	4.00	3.70
C. jejuni PEN 3	5.70	3.47	>4.70	>4.70

[a] All determinations for *C. jejuni* were done in duplicate; for *E. coli*, six replicate determinations were made.
[b] Greater than sign indicates that, under the experimental condition tested, no organisms were recovered on subculture of the lowest dilution.
[c] ND, Not determined.

TABLE 2. Effect of 1.0 mg of monochloramine per liter on three *C. jejuni* strains and *E. coli* ATCC 11229

Temp, pH, and strain	Initial bacterial count (log_{10} CFU/ml)	−(Log_{10} surviving fraction)[a] at:			
		5 min	15 min	30 min	60 min
25°C, pH 6					
E. coli ATCC 11229	5.05	0.29	2.84	3.27	4.44
C. jejuni PEN 1	6.13	ND[b]	4.83	ND	5.13
C. jejuni PEN 2	5.33	ND	>4.33	>4.33	>4.33
C. jejuni PEN 3	4.48	>3.48[c]	>3.48	>3.48	ND
25°C, pH 8					
E. coli ATCC 11229	5.07[a]	−.04	1.05	3.07	3.20
C. jejuni PEN 1	6.13	ND	4.65	ND	4.29
C. jejuni PEN 2	5.33	ND	>4.33	>4.33	>4.33
C. jejuni PEN 3	4.48	>3.48	>3.48	>3.48	ND
4°C, pH 6					
E. coli ATCC 11229	4.68[a]	0.11	0.75	1.61	2.47
C. jejuni PEN 1	5.72	ND	4.24	>4.72	>4.72
C. jejuni PEN 2	5.36	ND	3.36	>4.36	>4.36
C. jejuni PEN 3	4.11	>3.11	>3.11	>3.11	ND
4°C, pH 8					
E. coli ATCC 11229	4.68[a]	0.08	0.10	0.37	1.32
C. jejuni PEN 1	5.72	ND	2.80	3.88	>4.72
C. jejuni PEN 2	5.36	ND	3.58	3.22	3.88
C. jejuni PEN 3	4.11	>3.11	>3.11	>3.11	ND

[a] Results are based on 2 to 6 replicate determinations.
[b] ND, Not determined.
[c] Greater than sign indicates that, under the experimental condition tested, no organisms were recovered on subculture of the lowest dilution.

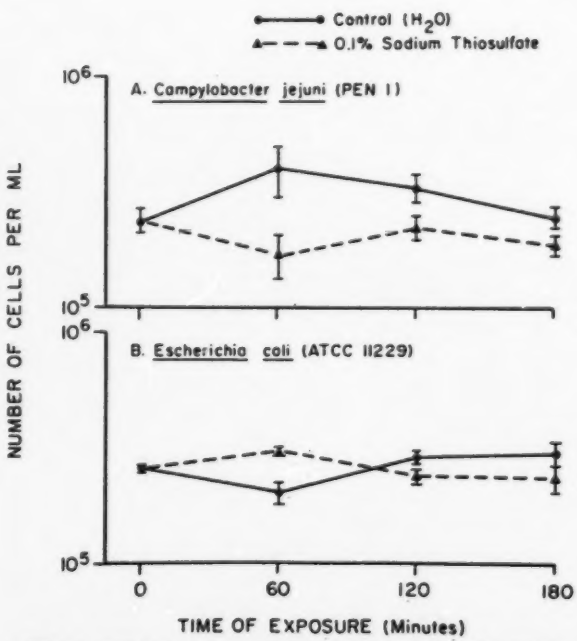

FIG. 1. Survival of *C. jejuni* PEN 1 and *E. coli* ATCC 11229 in 0.1% sodium thiosulfate at 5°C and pH 7.0. Points shown are means ± the standard errors of the means from triplicate determinations.

Monochloramine disinfection. Monochloramine demand produced by inoculation of test bacterial cells into the assay mixture was approximately 0.2 mg/liter after 3 h as determined by the DPD method. For both the *E. coli* and *C. jejuni* strains, disinfection with 1.0 mg of monochloramine per liter was more rapid at pH 6 than at pH 8 and at 25°C than at 4°C, as is expected from principles of chlorine chemistry (Table 2). In these experiments, we examined contact times ranging from 5 to 120 min. Within the first time point studied (5 or 15 min), 1 mg of monochloramine per liter resulted in greater than 99% inactivation of each of the *C. jejuni* strains studied under every condition. The difference in susceptibility between *C. jejuni* and *E. coli* was even more marked than in the chlorine studies; *C. jejuni* inactivation was more rapid or more complete or both under most conditions studied. Contact with the control buffer solutions alone produced no significant killing of any organism.

Inactivation of *C. jejuni* cells directly from mice. All the preceding experiments were done with *C. jejuni* strains that had been passed from two to six times on laboratory media. To control for the effects of passage on artificial media, we studied chlorine inactivation of *C. jejuni* cells directly from an animal source. Previous experiments had shown that the bacterial pellet originating from mouse feces produces chlorine demand from 0.3 to 0.4 mg/liter. In these experiments, initial free chlorine levels were high but, after 300 s of contact, fell to less than 0.10 mg/liter (Fig. 2). At 25°C and pH 6 or 8, fecal suspensions with 9×10^5 *C. jejuni* CFU/ml were rapidly inactivated in the presence of free chlorine (Fig. 2), but not in the buffer control (data not shown). The mouse aerobic bacterial enteric flora was inactivated less rapidly at both pHs 6 and 8. Identical experiments done at 4°C instead of 25°C showed less rapid inactivation, but again *C. jejuni*

TABLE 3. Comparison of use of a nonselective medium and an antibiotic-containing medium on the recovery of *C. jejuni* PEN 1 after contact with 0.1 mg of free chlorine per liter at 25°C

pH	Contact time (s)	Medium	Viable cells (CFU/ml)[a]	$-(\text{Log}_{10}$ surviving fraction)[b]	Log_{10} difference
6	30	BAP	1.2×10^3	1.77	
6	30	CBAP	5.0×10^1	3.15	1.38
6	300	BAP	2.8×10^2	2.40	
6	300	CBAP	1.0×10^1	3.85	1.45
8	30	BAP	4.9×10^3	1.16	
8	30	CBAP	1.9×10^3	1.58	0.42
8	300	BAP	2.4×10^2	2.46	
8	300	CBAP	7.0×10^1	3.00	0.54

[a] Mean of duplicate determinations.
[b] From original inoculum of 7.0×10^4 CFU/ml.

levels fell to a greater extent than did those of the background fecal flora (data not shown).

Effects of antibiotic-containing media. In other experiments, we also compared the effect of nonselective and antibiotic-containing media on the recovery of *C. jejuni* PEN 1 cells after disinfection (Table 3). At pH 6, there was greater than a 1 $\log_{10}$ difference in the numbers recovered after either 30 or 300 s of contact time. At pH 8, the difference was about 0.5 $\log_{10}$; in each case, fewer *C. jejuni* cells were recovered from antibiotic-containing medium.

DISCUSSION

Chlorine and related halogens have been widely used for disinfecting drinking water for nearly 100 years. The standard disinfection levels used for chlorine and monochloramine are based on the susceptibility of *E. coli* and other indicator organisms (11, 13). Since *C. jejuni* may survive for extended periods in water (4) and recently has been recognized as a major waterborne pathogen, it is reasonable to ask whether standard levels of chlorine and monochloramine are sufficient for eliminating these organisms. We studied these disinfectants under stringent conditions. The contact times studied were shorter, and the halogen concentrations were lower than the standards recommended for water treatment (11, 13), and the *C. jejuni* concentrations were far higher than are likely to be seen, even in grossly contaminated waters (4, 17). Therefore, we are reasonably confident that susceptibility results in the laboratory would translate into safety under field conditions.

We found that, under the experimental conditions studied, the three *C. jejuni* strains were as susceptible to the bactericidal effects of chlorine as was the *E. coli* test strains or more so. These results indicate that chlorination procedures that effectively inactivate *E. coli* from water also will be effective for inactivating *C. jejuni*. Since the reservoir in nature for *C. jejuni* is feces, in fecally contaminated water the absence of *E. coli* after chlorine disinfection should also suggest absence of *C. jejuni*. In general, increasing water pH from neutrality decreases germicidal efficiency, because most of the free chlorine exists as the less microbicidal OCl^- at the higher pHs. For *E. coli*, HOCl is more than 50-fold more effective than OCl^- as a disinfectant (16), and for *C. jejuni* a similar phenomenon was observed.

C. jejuni strains recovered directly from mouse feces or laboratory passaged were inactivated to similar degrees for each condition tested These experiments confirm that laboratory-passaged strains are not more susceptible to chlorine inactivation than are strains isolated directly from animals, and they further illustrate that *C. jejuni* is more susceptible to chlorine disinfection than is other fecal flora which is less pathogenic to humans.

Our studies were designed to measure inactivation of *C. jejuni* as determined by decreases in the number of viable cells on nonselective media. However, we also were able to detect cells with sublethal injury (12) by comparing viable counts on a nonselective medium with those on a selective (antibiotic-containing) medium (19). In addition to killing of *C. jejuni* cells by free chlorine, we also found that a sizable proportion of those remaining were injured, especially at pH 6. This phenomenon presumably increases still further the margin of safety of chlorine disinfection of *C. jejuni*.

Monochloramine disinfection is slower and requires higher concentrations than for free chlorine (11, 13). Butterfield and colleagues (9, 10) found that, for 100% inactivation of *E. coli* by chloramines in 60 min, at pH 7.0 0.6

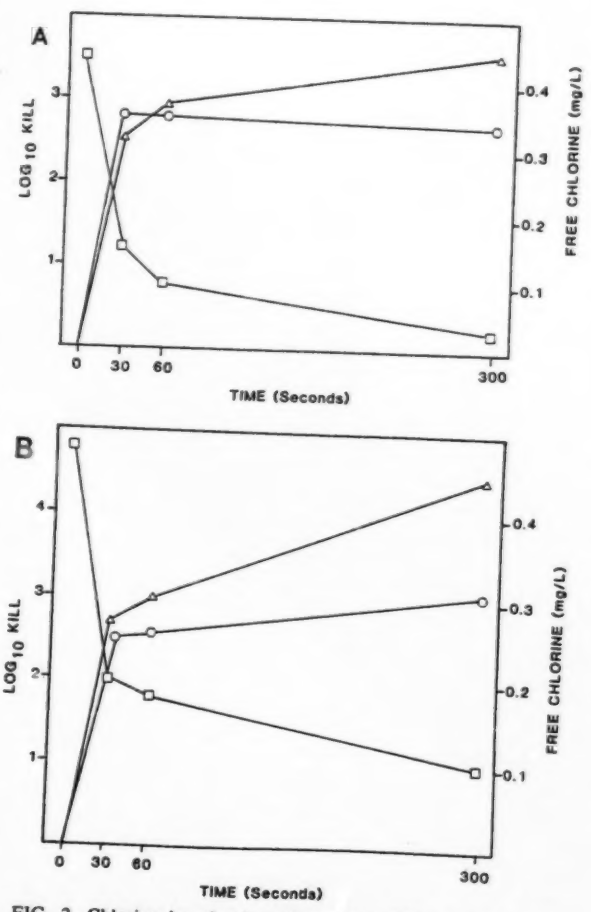

FIG. 2. Chlorine inactivation of *C. jejuni* PEN 1 and aerobic mouse fecal flora at 25°C and pH 6 (A) or 8 (B). Feces from mice colonized with *C. jejuni* were collected, and the bacterial phase was separated and assayed for chlorine inactivation of *C. jejuni* (Δ) and aerobic fecal flora (○). To compensate for chlorine demand by the bacterial cells, initial free chlorine levels were 0.44 to 0.48 mg/liter. Free chlorine (□) was measured throughout the assay. Each point shown represents the mean of duplicate data points.

mg/liter was required, and at pH 8.5 1.8 mg/liter was required. No distinction between monochloramine and dichloramine was made. D. L. Siders, P. V. Scarpino, M. Lucas, G. Berg, and S. L. Chang, (Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, E27, p. 5), using 1.0 mg of monochloramine per liter alone, found that 99% (2 $\log_{10}$) inactivation of *E. coli* occurred in 20 min at 15°C and pH 9. Although our experimental methods differed from those used by those investigators, our data for inactivation of *E. coli* are consistent with theirs. As such, our finding that the three *C. jejuni* strains studied were more rapidly and completely inactivated than the *E. coli* strain under every condition tested demonstrates the efficacy of present monochloramine disinfection standards for removing these pathogens.

These results confirm our previous findings in disinfectant studies, in which the assay conditions were not specifically designed to mirror those found in water treatment plants (18). Similarly, in earlier work in which chlorine demand was present during the assay, we found a similar relationship between *E. coli* and *C. jejuni* susceptibilities to chlorine (8). Further, these bacteriologic results support the epidemiologic association that waterborne infections due to *C. jejuni* occur only if humans ingest untreated or improperly treated water. The absence of *C. jejuni* outbreaks associated with consumption of properly treated water is consistent with our finding of marked susceptibility of these organisms to the standard procedures for disinfection of drinking water.

ACKNOWLEDGMENTS

This work was supported by cooperative agreement no. R81-0169 from the Office of Research and Development, U.S. Environmental Protection Agency, and by the Medical Research Service of the Veterans Administration.

We thank Ronald G. West for his assistance in the initial phases of this project.

LITERATURE CITED

1. **American Public Health Association.** 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.
2. **Berman, D., and J. C. Hoff.** 1984. Inactivation of simian rotavirus SA11 by chlorine, chlorine dioxide, and monochloramine. Appl. Environ. Microbiol. **48**:317–323.
3. **Blaser, M. J., D. J. Duncan, G. H. Warren, and W.-L. L. Wang.** 1983. Experimental *Campylobacter jejuni* infection of adult mice. Infect. immun. **39**:908–916.
4. **Blaser, M. J., H. L. Hardesty, B. Powers, and W.-L. L. Wang.** 1980. Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. J. Clin. Microbiol. **11**:309–313.
5. **Blaser, M. J., and L. B. Reller.** 1981. *Campylobacter* enteritis. N. Engl. J. Med. **305**:1444–1452.
6. **Blaser, M. J., D. N. Taylor, and R. A. Feldman.** 1983. Epidemiology of *Campylobacter jejuni* infections. Epidemiol. Rev. **5**: 157–176.
7. **Blaser, M. J., J. G. Wells, R. A. Feldman, R. A. Pollard, and J. R. Allen.** 1983. *Campylobacter* enteritis in the United States. A multicenter study. Ann. Intern. Med. **98**:360–365.
8. **Blaser, M. J., R. G. West, W.-L. L. Wang, and J. C. Hoff.** 1984. Control of waterborne *Campylobacter jejuni* by chlorine disinfection, p. 287–294. *In* Water quality and treatment: advances in laboratory technology. Proceedings of the AWWA Water Quality Technical Conference. American Water Works Association, Denver, Colo.
9. **Butterfield, C. T.** 1948. Bactericidal properties of chloramines and free chlorine in water. Public Health Rep. **63**:934–940.
10. **Butterfield, C. T., E. Wattie, S. Megregian, and C. W. Chambers.** 1946. Influence of pH and temperature on survival of coliforms and enteric pathogens when exposed to free chlorine. Public Health Rep. **58**:1837–1866.
11. **Hoff, J. C., and E. E. Geldreich.** 1981. Comparison of the biocidal efficiency of alternative disinfectants. Am. Water Works Assoc. J. **73**:40–44.
12. **McFeters, G. A., S. C. Cameron, and M. W. LeChevallier.** 1982. Influence of diluents, media, and membrane filters on detection of injured waterborne coliform bacteria. Appl. Environ. Microbiol. **43**:97–103.
13. **National Academy of Sciences.** 1980. Disinfection of drinking water, p. 5–137. *In* Drinking water and health, vol. 2. National Academic Press, Washington, D.C.
14. **Penner, J. L., and J. N. Hennessy.** 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. **12**:732–737.
15. **Peterson, D. A., T. R. Hurley, J. C. Hoff, and L. G. Wolfe.** 1983. Effect of chlorine treatment on infectivity of hepatitis A virus. Appl. Environ. Microbiol. **45**:223–227.
16. **Scarpino, P. V., G. Berg, S. L. Chang, D. Dahling, and M. Lucas.** 1972. A comparative study of the inactivation of viruses in water by chlorine. Water Res. **6**:959–965.
17. **Taylor, D. N., K. T. McDermott, J. R. Little, J. G. Wells, and M. J. Blaser.** 1983. *Campylobacter* enteritis from untreated water in the Rocky Mountains. Ann. Intern. Med. **99**:38–40.
18. **Wang, W.-L. L., B. W. Powers, N. W. Luechtefeld, and M. J. Blaser.** 1983. Effects of disinfectants on *Campylobacter jejuni*. Appl. Environ. Microbiol. **45**:1202–1205.
19. **Wang, W.-L. L., N. A. Wilson, L. B. Reller, and M. J. Blaser.** 1980. Selective methods for isolation of *Campylobacter fetus* subsp. *jejuni*, p. 940–942. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease, vol. 2. American Society for Microbiology, Washington, D.C.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.